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CASE DB13NP

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Keith R. Lange
Type or print name

Signature

December 4, 2002
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
STARLING ET AL.
APPLICATION NO: 09/745,605
FILED: DECEMBER 22, 2000
FOR: NOVEL IMMUNOGLOBULIN SUPERFAMILY MEMBERS OF APEX-1, APEX-2 AND APEX-3 AND USES THEREOF

Art Unit: 1644
Examiner: Haddad, Maher M.

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF PRIOR INVENTION IN THE UNITED STATES TO OVERCOME A REFERENCE UNDER 37 C.F.R. § 1.131

Sir:

1. We, Gary C. Starling and Joshua N. Finger, both citizens of the United States, residing respectively at 11 Gallo Court, Lawrenceville, NJ 08648 and 197 North Union Street, Apt. B, Lambertville, NJ 08530 are joint inventors of the above-identified application.

2. At the time of the invention thereof we were working for Bristol-Myers Squibb Company, assignee of the present application. We submit this declaration to establish completion of the invention set forth in this application in the United States at a date prior to December 9, 1999, i.e. the publication date of WO 99/63088 to Baker et al. (hereinafter the '088 publication), which was cited by the Examiner in an Office Action mailed June 5, 2002.

3. From the documents submitted herewith and as set forth hereinbelow, it can be seen that the invention was completed in the United States before December 9, 1999, the publication date of the '088 publication. Completion of the invention prior to December 9, 1999 is shown by conception and actual reduction to practice of the invention as evidenced by the cloning and sequencing of the APEX-1 gene (hereinafter "APEX-1"), which is also referred to in Exhibit A as DCS4.

4. To establish conception and reduction to practice, i.e. completion of the invention at a date prior to December 9, 1999, the following documents are submitted as evidence:

a. Bristol-Myers Squibb Notebook No. 42973 assigned and completed prior to December 9, 1999 (Exhibit A), pages 42973-103 through 42973-106, 42973-112 through 42973-114, 42973-127 through 42973-129 and page 42973-158. These pages show the full-length cloning of APEX-1 and set forth the nucleotide and amino acid sequences of APEX-1, which correspond to SEQ ID NOS. 1 and 4, respectively, in the present application. The full length cDNA sequence and amino acid translation of APEX-1 is shown on page 42973-158. These notebook records evidence conception and actual reduction to practice of the complete invention prior to December 9, 1999.

5. The materials submitted herewith establish that the invention as claimed was completed, i.e. conceived and reduced to practice, at a date prior to December 9, 1999, the publication date of the '088 publication.

6. This declaration is submitted in a response to a non-Final Office Action dated June 5, 2002 and is therefore believed to be timely filed.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED:_____ _____
Gary C. Starling

DATED:_____ _____
Joshua N. Finger

EXHIBIT A

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

BRISTOL-MYERS SQUIBB

NOTEBOOK Nº 42973

Assigned to Joshua N. Jaeger

Department Name _____

Department Number _____

Date Assigned _____

Date Completed _____

Pages Completed from 001 to 200

Continued from Notebook Number _____

Continued in Notebook Number _____

This notebook cannot be transferred to another person

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* USE THE MOST APPROPRIATE DESCRIPTION, E.G.: COMPOUND NUMBER, PRODUCT NAME AND LOT NUMBER, GENERIC NAME, CHEMICAL NAME, CULTURE NUMBER.

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		" transformation	122
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* USE THE MOST APPROPRIATE DESCRIPTION, E.G.: COMPOUND NUMBER, PRODUCT NAME AND LOT NUMBER, GENERIC NAME, CHEMICAL NAME, CULTURE NUMBER.

DATE PROJ. NO. EXPT. NO.

SUBJECT Isolation of Clone #4 and Clone #82

Purpose: To isolate the cDNA inserts from clone #4 and clone #82 from plasmid DNA for use as a probe.

Materials and Methods

a) Clone #4

React III	0.1 ml
EcoR1	1 ml
dH_2O	3 ml
	10 ml

b) Clone #82

	19 ml
React III	3 ml
Pst I	1.5 ml
HindIII	1.5 ml
dH_2O	5.0 ml
	30.0 ml

Results:

[redacted]

Lane 1: λ/Ø Marker
Lane 2: Clone #4
Lane 3: ~~Clo~~ BLANK LANE
Lane 4 BLANK LANE
Lane 5 Clone #82
Lane 6 Clone #82

Discussion: Clone #4 must have a mutated EcoR1 site, cut again with HindIII and PstI. Go ahead and isolate clone #82 insert and analyze.

END

SIGNED DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: DATE

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT Re-digest of Clone #4 using PstI or HindIII

PURPOSE To drop out insert of clone #4 using enzymes other than EcoRI

Materials and Methods

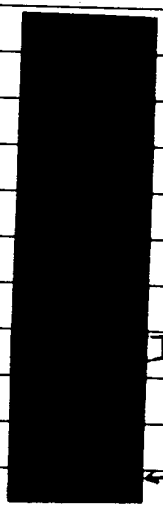
Clone #4	1.5 ul
Buffer II	3 ul
PstI	1.5 ul
HindIII	1.5 ul
dH_2O	9.0 ul
	30.0 ul

Results.

Lane 1 Φ marker

Lane 2 H/P digest of DSS4

Lane 3 H/P digest of DSS4



] Gel purify this fragment

← 2nd fragment

Discussion

Just as I suspected DSS4 clone has a mutated EcoRI site as shown by inability of EcoRI to cut out insert (42973-103, Lane 2). However, HindIII and PstI dropped out two fragments. One fragment approximately 400 bp in size will be gel purified and used as a probe (HP400). The 2nd fragment is approximately 80-90 bp and will go into the trash.

END

SIGNED _____ DATE _____

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: _____ DATE _____

DATE PROJ. NO. EXPT. NO.

SUBJECT Gel purification of clones #5 and clone #4

PURPOSE To gel purify both clones isolated from Dexthi cell subtraction library for use as probes in further expt.

5

Materials & Methods

See protocol from QIAquick Spin Handbook "QIAquick gel extraction kit" (QIAGEN Cat # 28704)

10

RESULTS

	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml
1)	0.0048	0.0027	0.0002	1.8457	0.5418	0.0025	23.1278
2)	0.0109	0.0045	-0.0006	2.2434	0.4457	0.0051	57.5225
3)	0.3912	0.2485	0.0023	1.5793	0.6332	0.2463	1944.6359
4)	0.4535	0.2929	0.0039	1.5558	0.6428	0.2890	2248.1201

$[\,] = (A_{260} \times (50^{ug}/_{ml}) \times D) / 1000 = {}^{g}/_{dl}$

1) DSS4

25 $(0.0048 \times 50^{ug}/_{ml} \times 25)/1000 = \boxed{6\ ^{ng}/_{ml}}$

2) DSS82

$(0.0109 \times 50^{ug}/_{ml} \times 25)/1000 = \boxed{13\ ^{ng}/_{ml}}$

3) MALIPEED DSS4 3) DSS4.1 $(0.3912 \times 50^{ug}/_{ml} \times 200)/1000 = \boxed{3.9\ ^{ug}/_{ml}}$

4) DSS4.2 $(0.4535 \times 50^{ug}/_{ml} \times 200)/1000 = \boxed{4.5\ ^{ug}/_{ml}}$

DISCUSSION:

35 NONE

END

SIGNED DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY Hastie DATE

NO. 42913-100

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE: 01, PROJ. NO. EXPT. NO.

SUBJECT: Southern Hybridization to confirm expression of PSS4 in subtracted library

PURPOSE: To analyze the presence of DSS4 in DI2 cDNA, THP1 cDNA and DI2-THP1 subtracted cDNA. To also see if other housekeeping genes such as G3PDH were subtracted out of the subtracted cDNA library.

Materials and Methods:

10ug of each cDNA was loaded into each well and ran @ 70Volts for 3 hours. DNA was stained (like gel photo)

	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml
1)	0.6093	0.4039	0.0198	1.5350	0.6515	0.3840	2947.4448
2)	0.9419	0.5982	0.0306	1.6055	0.6229	0.5676	4556.4517
3)	0.2023	0.1188	0.0005	1.7056	0.5863	0.1183	1008.9186

[ ] = Abs @ 260 nm × 50 ug/ml × D = ug/ml

1) DI2 cDNA:

0.4039 × 50 ug/ml × 50 = 1.5 mg/ml

2) THP1 cDNA: 0.9419 × 50 ug/ml × 50 = 2.4 mg/ml

3) DI2-THP1 subtracted cDNA: 0.2023 × 50 ug/ml × 50 = c.5 ug/ml

[redacted]

At Lane 1: DI2 cDNA

Lane 2: THP1 cDNA

Lane 3: DI2-THP1 subtracted cDNA

SIGNED: [signature] DATE:

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: [signature] DATE:

NO. 42913-116

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE: _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT: 3' RACE using DSS4 gene specific primer and QT, QI, QO

Purpose: To amplify the remaining 3' end of the unknown DSS4 gene which shows homology to CD84.

Materials and Methods

1) cDNA synthesis in Daudi, THP1, and Jurkat cells

poly A+ RNA	2 ul
Primer QT (INF3)	1 ul
dH2O (DEPC)	9 ul
	12 ul

*Incubate @ 70°C for 10 min. Ice 1 min.

Add to RNA/primer mix:

10x PCR Buffer	2 ul
25 mM $MgCl_2$	1 ul
10 mM dNTP's	2 ul
0.1 M DTT	2 ul
	7 ul

Incubate @ 42°C 5 min

Add 1 ul Superscript II RT

Incubate @ 42°C 1 hour

Stop Rxn by 70°C incubation

Add 1 ul RNase H

Store @ -20°C

continued 113

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SIGNED: [signature] DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: [signature] DATE

DATE PROJ. NO. EXPT. NO.

SUBJECT Continued from 42973-112

Continuation from 42973-112

2) Primary PCR using JNF1 and Q0(JNF4)

JNF1	1 µl
JNF4	1 µl
dNTP's	1 µl
10 x PCR Buffer	5 µl
~~T~~ cDNA	1 µl
dH_2O	39 µl
50x cDNA Advantage polymerase	1 µl
	50 µl

PCR parameters: 94°C 1min 1cycle
94°C 1min
57°C 1min } 25cycles
72°C 2min
72°C 2min 1cycle

3) Secondary PCR using JNF2 and Q- (JNF5)

JNF2	1 µl
JNF5	1 µl
dNTP's	1 µl
10x PCR Buffer	5 µl
1/50 dilution of 1° PCR	1 µl
dH_2O	39 µl
50x Polymerase mix	1 µl
	50 µl

PCR parameters: Same as Primary PCR

Continue to 114

SIGNED DATE

WITNESSED AND UNDERSTOOD BY: DATE

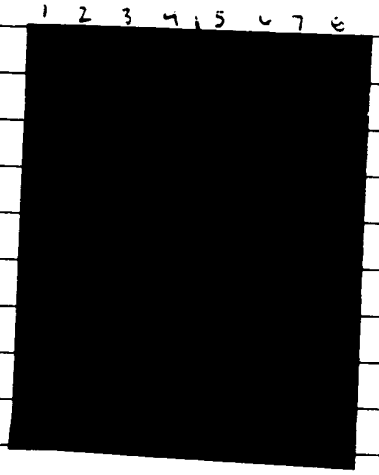
CROSS REFERENCES:

DATE ___ PROJ. NO. ___ EXPT. NO. ___

SUBJECT Continued from 42973-113

RESULTS

1 2 3 4 5 6 7 8



Lane 1 MW Marker
Lane 2 Dendritic 4-1
Lane 3 THP1 4-1
Lane 4 Jurkat 4-1
Lane 5 Dendritic 2-5
Lane 6 THP1 2-5
Lane 7 Jurkat 2-5
Lane 8 Scrap Marker

DISCUSSION

Lanes 2,3,4 on primary PCR using gapi(JNF1) and primer C6(JNF4). There seems to be background problems. Either extension time of 2 min was too long or Tm of annealing was low. Secondary PCR using gapi2(JNF2) and primer C5(JNF5) reduced background considerably, but contains several bands which may be nonspecific(Lanes 5,6,7). Continue with RACE experiments, starting with 1/50 dilution of 1° PCR, but increase Tm to 58°C and reduce extension to 1min(since most prominent band in Jurkat Lane is around 900 bp).

END

SIGNED John N. Jin DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY. Hack DATE

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DATE: PROJ. NO. EXPT. NO.

SUBJECT: Cloning of Full Length DC54 cDNA including 3'-UTR.

Purpose: Isolate The full length DC54 cDNA of size approximately 2.6 Kb from 50 µl of PCR mixture.

5 Materials and Methods:

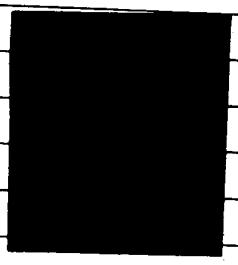
PCR

FP (Hieve11,2)	2.5 µl
RP (TNF4)	1.0 µl
dNTP's	1.0 µl
10x PCR Buffer	5.0 µl
Advantage Polymerase	1.0 µl
dH_2O	38.5 µl
Dendritic cDNA	1.0 µl
	50.0 µl

PCR parameters

94°C 1 min
94°C 1 min
57°C 1 min } 30
72°C 2.5 min
72°C 10 min

Run all 50 µl on 1.2% agarose gel.
Isolate 2.5 Kb band using gel extraction kit (QIAgen)
Run on 2.0% gel; check conc.



Lane 1: λØ marker
Lane 2: 1 µl isolated PCR fragment
Lane 3: 3 µl isolated PCR fragment
Lane 4: 2 µl Ø marker (1.3 Kb band = 16 ng)
Lane 5: 4 µl Ø marker (1.3 Kb band = 32 ng)

Conc. of isolated fragment is 100 : ≈2 ng/µl

Ligate fragment into TA cloning vector

Results: Amount of 2.5 Kb fragment isolated is very low, but I ligated as fast to try and isolate a clone.

Discussion: No discussion yet, wait until transformation.

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CROSS REFERENCES:

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BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE: PROJ. NO. EXPT. NO.

SUBJECT: RT-PCR using primers INF6 and INF7

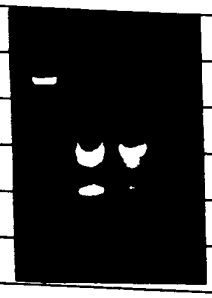
Purpose: To optimize parameters for RT-PCR expt, so as to get one band at 600 bp enriched in cell lines which express the DC-SIGN gene.

5 Materials and Methods

1) 1st PCR:

INF6 primer	1 ul	cDNA's used:
INF7 primer	1 ul	a) Dendritic
dNTP's	1 ul	b) LPS Nint T cell
10x PCR Buffer	5 ul	c) THP1
Advantage Polymerase	1 ul	
dH_2O	40 ul	
DNA	1 ul	
	50 ul	

Parameters: 94°C 1 min
94°C 1 min
61°C 1 min } 30 cycles
72°C 45 sec
72°C 1 min



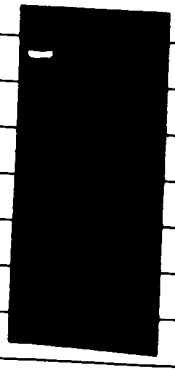
Lane 1: λ/ϕ marker
Lane 2: Dendritic
Lane 3: LPS Nint T
Lane 4: THP1

RESULT 1: 600 bp band was existed, but a high background was present in resultant. Redo PCR with elevation of annealing temp to 63°C, in order to reduce background.

2) 2nd PCR:

INF6 primer	0.5 ul	cDNA's used:
INF7 primer	0.5 ul	a) LPS Nint
dNTP's	0.5 ul	b) THP1
10x PCR Buffer	2.5 ul	
Advantage Polymerase	0.5 ul	
dH_2O	20.0 ul	
cDNA	0.5 ul	
	25.0 ul	

Parameters: 94°C 1 min
94°C 1 min
63°C 1 min } 25 cycles
72°C 45 sec
72°C 1 min



continue 42973-129

SIGNED: DATE:

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: DATE

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DATE _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT Continued 42973-128

Result 2: Significant reduction of background, providing evidence inhibition of uptake of bind in LPS Non T cell and no bind in THP1 as found earlier in ref 42973-125.

Discussion: This expt. was done to optimize PCR parameter in RT-PCR of immunological cell lines, in order to identify which cell type express the DCSH transcript. As shown above, LPS Non-T cells express DCSH, but THP1 does not. The significance of lack of expression in THP1 mRNA shows the original subtraction expt. up-regulated cDNA's expressed differentially between GMCSF/IL4 differentiated human monocytes and THP1 monocytes. This is also proven by expression of DCSH in dendritic cDNA. Repeat PCR for all available cell lines.

END

SIGNED _____ DATE _____

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: _____ DATE _____

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BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE PROJ. NO. EXPT. NO.

SUBJECT Full length cDNA sequence and a.a. translation for DC54

Word Document
FLDC54.doc

```
                                                GGAAGTGGCTTCATTTCAGTGGCTGACTTCCAGAGAGCAAT   41
ATGGCTGGTTCCCCAACATGCCTCACCCTCATCTATATCCTTTGGCAGCTCACAGGGTCA  101
M  A  G  S  P  T  C  L  T  L  I  Y  I  L  W  Q  L  T  G  S    20

GCAGCCTCTGGACCCGTGAAAGAGCTGGTCGGTTCCGTTGGTGGGGCCGTGACTTTCCCC  161
A  A  S  G  P  V  K  E  L  V  G  S  V  G  G  A  V  T  F  P    40

CTGAAGTCCAAAGTAAAGCAAGTTGACTCTATTGTCTGGACCTTCAACACAACCCCTCTT  221
L  K  S  K  V  K  Q  V  D  S  I  V  W  T  F  N  T  T  P  L    60

GTCACCATACAGCCAGAAGGGGGCACTATCATAGTGACCCAAAATCGTAATAGGGAGAGA  281
V  T  I  Q  P  E  G  G  T  I  I  V  T  Q  N  R  N  R  E  R    80

GTAGACTTCCCAGATGGAGGCTACTCCCTGAAGCTCAGCAAACTGAAGAAGAATGACTCA  341
V  D  F  P  D  G  G  Y  S  L  K  L  S  K  L  K  K  N  D  S   100

GGGATCTACTATGTGGGGATATACAGCTCATCACTCCAGCAGCCCTCCACCCAGGAGTAC  401
G  I  Y  Y  V  G  I  Y  S  S  S  L  Q  Q  P  S  T  Q  E  Y   120

GTGCTGCATGTCTACGAGCACCTGTCAAAGCCTAAAGTCACCATGGGTCTGCAGAGCAAT  461
V  L  H  V  Y  E  H  L  S  K  P  K  V  T  M  G  L  Q  S  N   140

AAGAATGGCACCTGTGTGACCAATCTGACATGCTGCATGGAACATGGGGAAGAGGATGTG  521
K  N  G  T  C  V  T  N  L  T  C  C  M  E  H  G  E  E  D  V   160

ATTTATACCTGGAAGGCCCTGGGGCAAGCAGCCAATGAGTCCCATAATGGGTCCATCCTC  581
I  Y  T  W  K  A  L  G  Q  A  A  N  E  S  H  N  G  S  I  L   180

CCCATCTCCTGGAGATGGGGAGAAAGTGATATGACCTTCATCTGCGTTGCCAGGAACCCT  641
P  I  S  W  R  W  G  E  S  D  M  T  F  I  C  V  A  R  N  P   200

GTCAGCAGAAACTTCTCAAGCCCCATCCTTGCCAGGAAGCTCTGTGAAGGTGCTGCTGAT  701
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D  P  D  S  S  M  V  L  L  C  L  L  L  V  P  L  L  L  S  L   240

TTTGTACTGGGGCTATTTCTTTGGTTTCTGAAGAGAGAGACAAGAAGAGTACATTGAA  821
F  V  L  G  L  F  L  W  F  L  K  R  E  R  Q  E  E  Y  I  E   260

GAGAAGAAGAGAGTGGACATTTGTCGGGAAACTCCTAACATATGCCCCCATTCTGGAGAG  881
E  K  K  R  V  D  I  C  R  E  T  P  N  I  C  P  H  S  G  E   280

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AATACGGTTTACTCCACTGTGGAAATACCGAAAAAGATGGAAAATCCCCACTCACTGCTC 1001
N  T  V  Y  S  T  V  E  I  P  K  K  M  E  N  P  H  S  L  L   320

ACGATGCCAGACACACCAAGGCTATTTGCCTATGAGAATGTTATCTAGACAGCAGTGCAC 1061
T  M  P  D  T  P  R  L  F  A  Y  E  N  V  I  *               335

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CAGTGAGCTGAGATGGCACCACTGCACTCCGGCCTAGGCAACGAGAGCAAAACTCCAATA 2321
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CATAAAAAAAAAAAAAAAAAAAA
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SIGNED DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: DATE